knee OA is controversial. However, recent findings indicate that there may be a relationship between muscle and incident or progressive symptoms and structural change in women but not in men. Yet, little is known about sex differences of muscle development at various stages of human development, particularly in relation to local bone size.

Methods: 20 young (baseline age 16.0±0.6y) and 20 mature (46.3±4.7y) top volleyball athletes were studied: 10 young men (BMI 22.3±0.9) and women (20.9±2.0), and 10 mature men (BMI 26±2.6) and women (22.7±1.9). The adolescent athletes trained twice per day (each session approx. 2 hours), and the former (mature) volleyball athletes trained about twice per week. Axial MR images (T1-weighed spin echo) of both thighs were acquired at baseline and 2 year follow-up, extending from the femoral neck proximally to the quadriceps tendon distally. Quadriceps muscle, total femoral and cortical bone CSAs were determined in the dominant leg (the one used for takeoff), in an MR image that was located 30% from proximal to distal (where quadriceps CSA was previously shown to correlate best with quadriceps volume). Differences between men and women and between adolescent and mature athletes were explored using unpaired t-tests, and longitudinal difference during the 2-year observation period using paired t-tests.

Results: At baseline, men had significantly ($p<0.001$) greater quadriceps, total femoral bone, and cortical femoral bone CSAs than women, both in adolescent and in mature athletes (Table 1).However, the ratio between quadriceps vs. total femoral (or cortical) femoral bone area did not differ significantly between men and women at either age (Table 1; $p>0.25$). Mature women and men had smaller quadriceps ACSAs than the adolescent men and women (Table 1), but the difference only reached statistical significance in the women ($p<0.05$). The ratio of quadriceps vs. total and cortical femoral bone area was significantly ($p<0.05$) smaller in mature than in adolescent athletes, both in men and women (Table 1). No difference in the percent cortical (of total) femoral area was seen between sexes or age groups (Table 1; $p>0.18$). No significant changes in quadriceps CSA were observed during the 2 year observation period, although the increase in adolescent men (5.0±7.1%) reached borderline significance ($p=0.08$). A significant increase in cortical (3.2±2.8%) and total femoral area (2.8±2.6%) was noted in adolescent men (both $p<0.01$), but not in adolescent women ($p>0.24$) or mature study athletes ($p>0.23$). The increase in quadriceps CSA in the adolescent men was associated with the increase in femoral cortical bone area (Pearson correlation coefficient $r=0.70$; 95%CI -0.02,0.94; $p=0.06$).

Table 1
Muscle and bone CSA in volleyball athletes (W = women; M = men): mean±SD at baseline

	Adoles. W	Adoles. M	Mature W	Mature M
Quadriceps (cm2)	67.7±9.9	87.2±7.5	59.6±5.3	80.3±7.6
Tot fem bone (cm2)	6.10±0.88	7.77±0.98	6.24±0.67	8.12±0.80
Radio (Quad/tot fem)	11.16±1.41	11.32±1.17	9.66±1.30	9.96±1.17
Cort fem bone (cm2)	4.80±0.63	6.11±0.66	5.03±0.40	6.40±0.63
% Cort/tot fem bone (%)	78.9±4.6	79.0±6.2	80.9±4.2	78.9±1.8

Conclusions: These findings reveal sex-specific muscle vs. bone relationships in active adults at different stages of maturation. It is important to note that the observed relationships may be specific to subjects with intense training and loading histories, and may not apply to less active individuals. The observed changes of muscle and bone tissue in adolescent men appear to be coupled, whereas no (more) change was observed in women at this stage of maturity. Although men obviously had larger quadriceps and bone CSAs than women, the ratio between muscle and bone tissue did not display sex-specific differences at either stage of maturity. The cross sectional results suggests that in adulthood (i.e. 30 years after adolescence) approx. 92% of the muscle mass of very well trained young men, and approx. 88% of that of young women, can be maintained when regular sportive activity is continued. Future studies need to explore to what extent these relationships may be relevant to the incidence and progression of symptoms and degenerative structural change in knee OA.

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THE DIFFERENCES ON EXTRACELLULAR MATRIX AMONG EACH PORTION OF MENISCUS

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Purpose: Meniscus consist of fibrochondrocytes embedded in an extracellular matrix composed of a hydrophilic proteoglycan gel enmeshed in dense network of type I collagen. However it is not clear that extracellular matrix in meniscus. Therefore the purpose of this study was to analyze differences in the extracellular composition and collagen orientation of the central and peripheral portions of the meniscus.

Methods: Six-month-old pigs were used for current study. The menisci were removed from the six knees. According to anatomical feature of meniscus, each meniscus was divided into six portions (central and peripheral portion of anterior, middle and posteria part). The histologic analysis, biochemical analysis, scanning electron microscope (SEM) and compression test were performed. After the samples were embedded in paraffin, they were sectioned to 6μm. The sections were stained with Hematoxylin/Eosin (HE), SafraninO/Fast Green, and Picrosirius Red. The samples were observed with SEM. For biochemical analysis, samples were digested by papain and HCL for DMMB assay and hydroxyproline assay. Glycosaminoglycan (GAG) and collagen content were quantified by using microplate reader. For biomechanical analysis, 1mm thick of samples were cut from six portions of meniscus. Then we tested the peak stress of 50% stain. All statistical analyses were performed by the two-sample t-test method.

Results: Blood vessel in peripheral portion was more than in central portion. Picrosirius Red stain showed higher density of collagen in peripheral portion. Furthermore some collagen fibers parallel to surface near the surface to femur and tibia were observed. There were some large collagen fibers in peripheral portion(Fig.1). Glycosaminoglycan was stained with red in Safranin-O/Fast Green in central portion obviously(Fig.2). As the result of Picrosirius Red stain, the higher density of collagen fiber in peripheral portion was seen by SEM observation(Fig.1). The layer of surface to femur and tibia were different with internal structure of meniscus. Most of collagen orientation of internal structure was parallel fibers which perpendicular to cut surface. However the collagen fibers in surface to femur and tibia were looser than internal collagen fibers. The parallel collagen layers were observed in surface area. In tibia side, some crimp construction was observed. DMMB assay showed that glycosaminoglycan content in central portion was more than in peripheral portion ($p < 0.05$). In accord with the result of Picsirius Red stain and SEM, more collagen content in the peripheral portion was quantitated by hydroxyproline assay ($p < 0.05$))(Fig.3). In compression test, the result reflected central portion was stronger against mechanical stimulation but only posterior portion of medial meniscus and middle portion of lateral meniscus were significant ($P < 0.05$).

Conclusions: Collagen content in peripheral portion was more than in central portion. On the contrary, glycosaminoglycan content in central content was more than in peripheral portion. IN this study, there are not significant differences among anterior, middle and posterior portion. The difference of extracellular matrix contents reflected the resistance against mechanical strength. These results may leads to understanding for biology of meniscus.

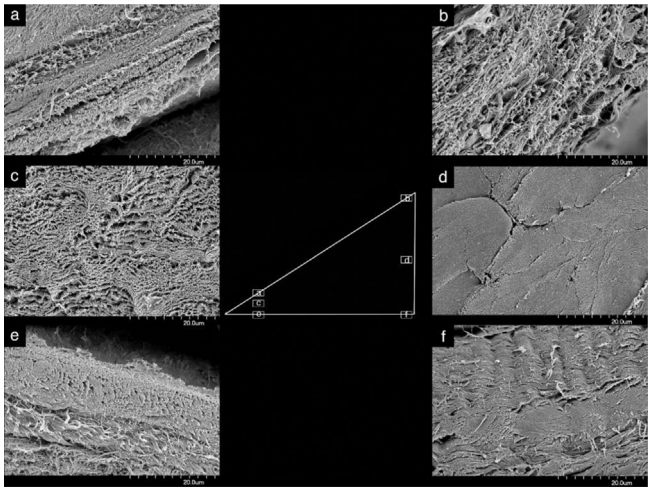

Fig.1, This is result of SEM of medial meniscus. In the middle layer, the density of collagen fibers in the peripheral portion was more than in central portion. In the surface, parallel collagen fibers could be observed.

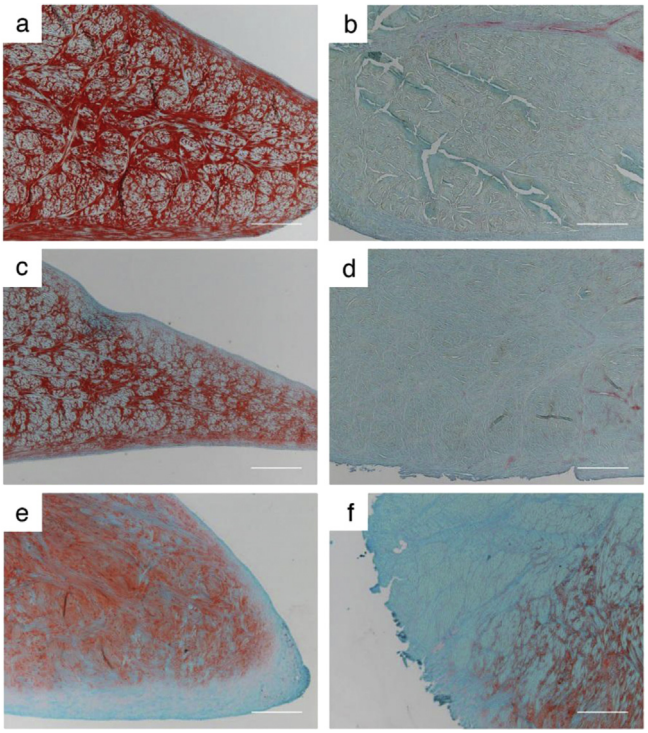

Fig.2, GAG was stained by Picrosirius Red in the central portion.

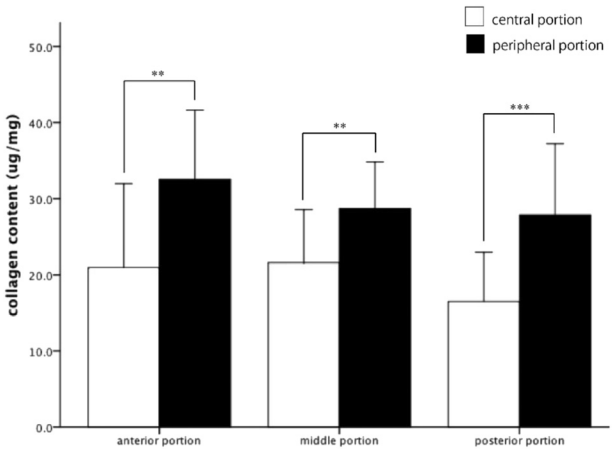

Fig.3, In hydroxyproline assay, collagen content in the peripheral portion was more than in the central portion.

miRNA

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MIR-193B-3P REGULATES CHONDROGENESIS OF ATDC5 CELLS VIA TARGETING TGFBR3

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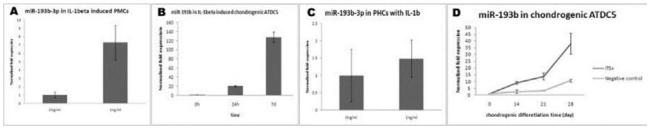
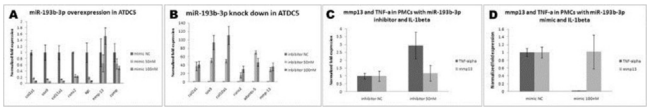
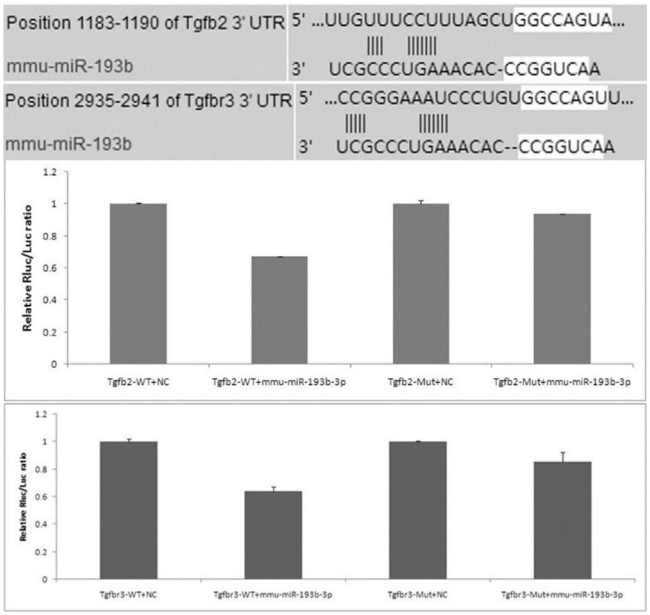
Purpose: To investigate the biological effect of mmu-miR-193b-3p on chondrogenic differentiation.
Methods: Chondrocyte-like ATDC5 cell line was stimulated with ITS+ premix to form cartilage nodules. Total RNA was isolated and reverse transcripted into cDNA. The 3'-UTR of predicted target genes, TGFBR3, were cloned into luciferase reporter plasmids. The mmu-miR-193b-3p mimic/inhibitor, and luciferase reporter plasmids were transfected into cells with lipofectamine 2000. Alcian blue were used to stain the cartilage nodules.
Results: The miR-193b expression was elevated in chondrogenic ATDC5.The miR-193b suppressed the expression of several chondrogenic markers in chondrogenic ATDC5 in a dose dependent manner, including col2a1, sox9, col10a1, col11a1, runx2, aggrecan, and comp. The mouse TGFBR3 were predicted as the potential target gene of mmu-miR-193b-3p.
The luminescence decreased more than 30% in 3T3 cells cotransfected with TGFBR3 3'-UTR reporter plasmids and miR-193b-3p mimic, while the mutation of predicted seed sequences of TGFBR3 3'-UTR partially restored the luminescence.
Conclusions: The miR-193b may inhibit chondrogenesis of ATDC5 via targeting TGFBR3.







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BOTH miRNA-29B DOWNREGULATION AND miRNA-140 OVEREXPRESSION DRIVE RESPECTIVELY MSC PROLIFERATION AND CHONDROGENIC DIFFERENTIATION IN COLLAGEN SCAFFOLD

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Purpose: MicroRNAs (miRNAs) play an important role in the regulation of chondrogenesis of human bone mesenchymal stem cells (hBMSC), however their respective expression during 2D expansion and 3D chondrogenic differentiation in collagen scaffold still remains poorly known. In this study, miRNA profile expressions during hBMSC chondrogenic differentiation was explored as putative biomarkers of chondrogenesis.
Methods: Mesenchymal stem cells issues from human bone marrow (hip replacement) were amplified and pre-conditioned by a specific medium (PAD) in the last passage (P3). Cells were then seeded in collagen sponges (Day 0) and cultured 28 days in a chondrogenic medium containing TGF-β1. The expression of miRNA was analyzed by hybridization on DNA microarrays then confirmed by qRT-PCR. The expression of these miRNA was compared with the extracellular matrix synthesis, and more particularly type II collagen and proteoglycan synthesis/content with qRT-PCR and histology respectively.